and then slowly withdrawn, leaving a thin layer of the diluted emulsion in the loop. The emulsion is then placed on the specimen grid by passing the loop down over the stationary grid.

After coating, the secured grids are placed in a light-tight box and exposed for a suitable length of time at 4°C. With plutonium-239, the radioelement used in this study, 3 days of exposure were found to be sufficient. The autoradiograms are then developed by immersing the stainless steel block supporting the grids in Eastman Kodak D-19 developer for 5 minutes, rinsing twice in tap water, fixing in acid fixer for 15 minutes, and thoroughly rinsing in running tap water for 30 minutes. After drying, they are examined with an RCA EMU-2C electron microscope.

An example of the results obtained is shown in Fig. 2, which illustrates the differentiation of radioactive and nonradioactive particles (*5*).

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25 November 1960

Phototropic Inversion in Phycomyces

Abstract. Transient reversed bending follows an adequate step-up in light intensity given a bending cell from any direction around its axis, yet the plane of the response is specifically that in which prior phototropic bending occurred. The cell "remembers" what it was doing and reverses this in the ensuing light-growth response. Antecedent asymmetric growth itself built the pattern for reversal.

An upright sporangiophore of *Phycomyces* exposed to a single horizontal beam of visible light responds by bending toward the light source; this is its characteristic *positive* phototropism. Bending rate, measured as the change in angle between the cell's extremity and the vertical, is nearly constant over a period of 10 to 15 minutes, and over a wide range it is independent of the intensity (*1*). This implies that the differential growth underlying bending is primarily determined by the asymmetry of the illumination pattern around the cell's axis and not by the level of light intensity.

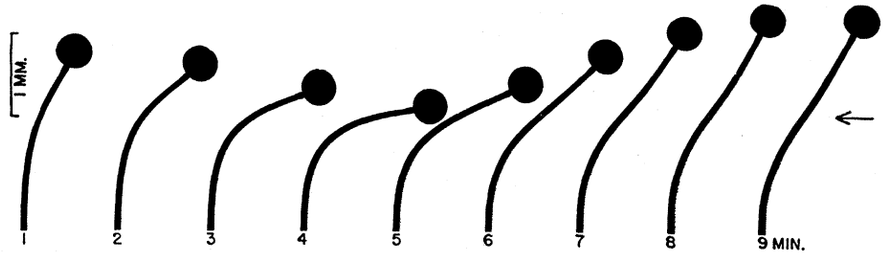

Fig. 1. Serial time-lapse photographs of *Phycomyces* showing normal bending interrupted by inversion. Abscissa, time; ordinate, vertical axis. Low intensity light source continuously illuminates cell from right (arrow); cell was straight when this commenced at −6 minutes. Normal positive bending is seen in pictures taken at 1 through 4 minutes. At 0 time the bending cell was given a 15-second flash of high intensity light from behind (perpendicular to plane of paper). Reversed bending is seen in pictures taken at 5 through 9 minutes; it takes place in the same vertical plane (that of the paper) as initial bending. Later (not shown) normal positive bending is restored.

Paradoxically, Reichardt and Varjú (*1*) showed that positive phototropic bending could be temporarily halted and reversed by a sudden sustained increase in the intensity of the horizontal stimulating beam. The established bending rate after a delay falls to zero and reverses its sign so that the cell for a time bends *away from* the light source; then this phase of *negative* phototropism ends, and the cell gradually regains its normal positive bending rate. This inversion is remarkable because it is caused simply by a properly timed increase in the intensity of the stimulating beam; the asymmetry of the illumination pattern remains unchanged.

I have studied inversion by photographic methods, previously described (*2*), that permit determination of both bending and cell elongation and have found that an acceleration of the cell's elongation rate indeed accompanies inversion. Reichardt and Varjú, however, always induced inversion by a step-up in intensity of the original stimulating beam, and their explanation assumes that an illumination pattern *within the cell* polarized in this direction and produced by the cell's lens action is necessary for inversion. This assumption is wrong. I have used a movable, supplementary light source to give the desired increase in light intensity along any chosen direction in the horizontal plane. I found that a sufficient step-up in intensity given even at right angles to the normal phototropic beam (or apparently at *any* angle to it) produces inversion (Fig. 1). So also does a 1-second flash of very high intensity light even though it is known to be phototropically ineffective and presumably saturating (*3*). Inversion is therefore caused by a step-up in light intensity *unspecific* as to both its direction and the cellular illumination pattern produced, even though the plane in which inversion occurs is *specifically* the plane of prior bending. Figuratively, the cell "remembers" what it was doing. This can only have a structural basis in the recent past history of the cell.

Growth requires supply systems and irreversibly forms the fibrils of the wall. Material so used comes from the substrate and more immediately from the cell's interior. The available local supply is the balance between rate of arrival and rate of use. I suppose that growth involves the presence of at least two materials, S and M. The supply of S is affected by light and tied to the adaptation system; M is used up in growth but is independent of light except as light affects its removal through use. Locally available M may be called growth *capacity*. The momentary rate of growth is considered proportional to the product $S \cdot M$. If more light is given, $S \cdot M$ temporarily rises, then declines in part because the growth capacity falls through the use of M. This is the light-growth response. With unilateral illumination $S \cdot M$ is kept slightly higher on the cell's far side by some consequence of the lens action. If subscripts n and f denote the near and far sides of the cell, the following conditions should apply during steady phototropic bending:

$$S_f \cdot M_f > S_n \cdot M_n$$
$$S_f > S_n$$
$$M_f < M_n$$

Thus local growth capacity, M, during steady bending is inversely related to the local growth rate, being high on the concave (near) side and low on the convex (far) side.

Such a bending cell is now given a step-up in light intensity: S rises rapidly wherever light acts and growth briefly accelerates, but then the ratio $S_f \cdot M_f / S_n \cdot M_n$ falls because M_f is now further depleted by fast growth; hence temporarily $S_n \cdot M_n$ can exceed $S_f \cdot M_f$ and *reversed* bending sets in. This is the inversion phenomenon, and it lasts until the level of M is restabilized in relation to S. The kinetics sought by Reichardt and Varjú involve the tem-

poral interplay between a light-sensitive system and a cooperating "dark" system.

The chief evidence for this interpretation lies in the cited irrelevance for inversion of the light pattern evoking it. A depletion effect is also suggested by the fact that reversed bending does not start until after 2 to 3 minutes of unusually rapid elongation; in fact, the maximum rate of reversed bending just about coincides in time with the maximum rate of elongation. Further, in studying the distribution of elemental curvature along the growth zone during bending and inversion, I found that the maximal initial rate of reversed bending occurs at or very near the locus of maximal curvature; this would be that level in the growth zone where, across the cell, the greatest accumulated difference in growth capacity exists, and where a burst of light ought to initiate the fastest reversed bending.

The spatial distribution of bending velocity changes with time in a way suggesting that capacity deficit first develops high in the growth zone because supply is basically from below (*4*). I attribute the strict polarization of reversed bending in the plane of prior curvature to the pattern of altered growth capacity established by the different growth rates during prior bending. This pattern may well involve different states of extension of the cell wall's microfibrils which are engaged in multinet growth (*5*).

Growth capacity evidently works with adaptation but overrides it under conditions where a burst of light unmasks latent capacity differences across the cell's diameter. One might choose to consider growth capacity as a normally concealed supply problem supplementary to adaptation. But operationally, adaptation is known only by what a system does: whether, how much, and in what direction it responds. Hence the scheme of Delbrück and Reichardt for the light responses of *Phycomyces* (*6*) must be judged imperfect. Its deficiency confounds Reichhardt and Varjú's analysis of inversion.

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9 January 1961

Nitrogen Deficiency and Fluoride Susceptibility of Bean Seedlings

Abstract. Groups of bean seedlings (*Phaseolus vulgaris*) were grown concurrently in cleaned and fluoride-containing (4 to 7 μg of F^- per cubic meter) air in plant-growth chambers for 10 and 20 days with six water-culture treatments: Hoaglund's formulation and Hoaglund's minus potassium, calcium, nitrogen, phosphorus, and iron. An inadvertent 15-hour exposure on day 15 (at 42 $\mu g/m^3$) produced foliar fluorosis symptoms only on the nitrogen-deficient plants fumigated 20 days, although higher fluoride concentrations were present in the foliage of similar plants grown in the other nutrient-deficient solutions.

The limited published data relating nutrition levels to susceptibility or resistance of plants to atmospheric fluoride exposure appear to be somewhat paradoxical. Brennan *et al.* (*1*) studied visible fluoride-induced, foliar toxicity in tomatoes as modified by alterations in the nitrogen, calcium, and phosphorous nutrition. They concluded that medium levels of nitrogen and calcium nutrition favored sorption of toxic quantities of fluorine, whereas low or deficient nutrition levels prevented fluorine injury. Fumigation concentrations of 48 and 470 parts of hydrogen fluoride per billion were used in these experiments.

Applegate and Adams (*2*) recently reported the effect of nutritional and water deficiencies on respiration and relative fluoride uptake of bean seedlings (*Phaseolus vulgaris*) at low fumigation levels of fluoride. Ten- and twenty-day fumigations were conducted at approximately 2 μg of F^- per cubic meter (1.6 parts per billion) of hydrogen fluoride, which is well below exposure levels required to produce visible, fluoride-induced foliar necrosis. The data showed that deficiencies in potassium, phosphorus, and iron accelerated the uptake of atmospheric fluoride. Direct comparison of data was impossible because of basic differences in the experimental techniques that were used in these separate investigations (*1*, *2*).

Studies at low fumigation concentrations are continuing in this laboratory. All plants are grown in an air pollution phytotron (*3*), and the experimental techniques have been described previously (*2*). Recently, one set of beans was inadvertently exposed to a high concentration of atmospheric hydrogen fluoride. For a period of 15 hours on day 15 of a 20-day exposure, the average concentration was 42 $\mu g/m^3$ (34 parts per billion). This acute exposure produced foliar necrosis in all plants subjected to one of the six nutrient treatments being studied. This report presents some observations on the influence of nutrition upon the susceptibility of beans to visible foliar injury from atmospheric fluorides (*4*).

One-half of the 20-day fumigated and control bean plants are shown in Fig. 1. The duplicate plants (not photographed) showed similar characteristic growth patterns and foliar markings. No fluorine-induced necrosis was observed in the group of 48 plants fumigated for 10 days and removed from the phytotron prior to the accidental 15-hour exposure at 42 $\mu g/m^3$. Foliar fluorosis was observed on all eight of the plants grown in nitrogen-deficient nutrient and fumigated 20 days. No fluoride-induced foliar symptoms were

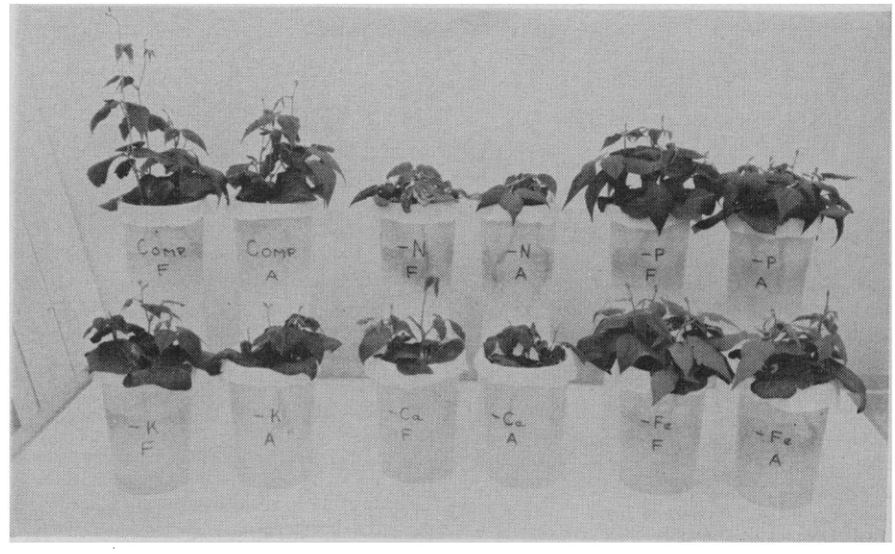

Fig. 1. Twenty-day fluoride-fumigated (F) and control (A) bean plants (*Phaseolus vulgaris*) grown in complete (Hoaglund's) nutrient solution "COMP" and nutrient solutions from which nitrogen (— N), phosphorus (— P), potassium (— K), calcium (—Ca), and iron (—Fe) have been withheld.